

Bird Goën & Co

1

C1564-US

DRAFT-CONFIDENTIAL

Declaration of Dr. Marc Jacquemin under 37 CFR § 1.132

1. I, Marc Jacquemin declare and state as follows. I am a professor at the University of Leuven and I am an expert in the field of vascular biology. I am one of the inventors of the U.S. Patent Application No. 10/030,522 entitled 'Ligands for use in therapeutic compositions for the treatment of hemostasis disorders'.
2. I understand that the Examiner has questioned, in the Office Action mailed on May 20, 2004, the fact that single chain variable parts of antibodies directed to the C1 domain of FVIII would be capable of binding FVIII and inhibiting FVIII activity.
3. I herewith present additional data which demonstrate that the single chain variable part of the antibody produced by the cell line Krix-1, which is directed to the C1 domain of FVIII, is capable of inhibiting FVIII activity.
4. In our laboratory, an scFv of KRIX-1 was constructed by adding a linker sequence between the 3' end of the KRIX-1 light chain variable part (VL) and the 5' end of the heavy chain variable part (VH), using standard technology known to the skilled person at the filing date of the present application. The technical details are provided herewith. The resulting scFv-KRIX-1VLVH was cloned into the pPICZαC expression vector and the final pPICZαC-scFv-KRIX-1VLVH(His) vector was used to transform X33 cells for scFv production. The supernatant was tested to demonstrate the presence of a functional scFv fragment. The scFv fragment was purified and after concentration, the scFvKRIX-1VLVH(His) was tested in a FVIII chromogenic assay to evaluate the ability of the scFvKRIX-1VLVH(His) to inhibit FVIII activity. The FVIII inhibitory capacity was evaluated in a Bethesda assay (described in the application as filed): one volume of buffer with scFvKRIX-1VLVH(His) at various concentrations was mixed with one volume of a pool of normal human plasma and incubated for 2h at 37°C. The residual FVIII activity was then measured in a chromogenic assay.
5. The results, presented in Figure 1 enclosed herewith, clearly demonstrate that scFv of the Krix-1 antibody has inhibitory activity on FVIII.
6. I hereby declare that all statements made herein are true and are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of the application or any patent issued thereon.

Date:

By: Dr. Marc Jacquemin

Bird Goen & Co

2
DRAFT-CONFIDENTIAL

C1564-US

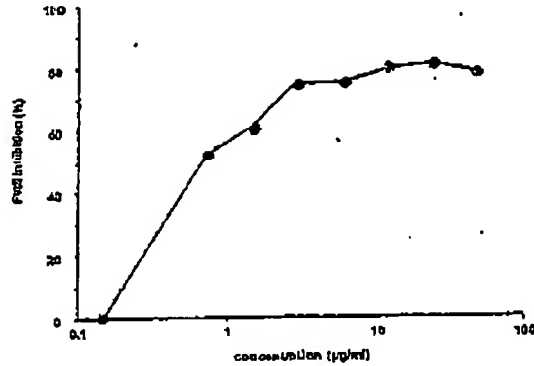
Figure 1.

Figure 1: Graph of experimental results showing the FVIII inhibitory activity of scFv fragment of KRIX-1 (scFv-KRIX-1VLVH(His)) produced in *Pichia pastoris*, in accordance with an embodiment of the invention.

Bird Goen & Co

3

C1564-US

DRAFT-CONFIDENTIAL

Technical details of the production and characterization of KRIX-1 scFv fragment**1. Cloning of scFv-KRIX-1VLVH in Pichia expression vector**

An scFv of KRIX-1 was constructed by adding a linker sequence between the 3' end of the KRIX-1 light chain variable part (VL) and the 5' end of the heavy chain variable part (VH). This was obtained by PCR amplification of KRIX-1 light chain and heavy chains using the following primers:

a) light chain
forward primer:

5'-gtatctctcgagaaaaga**GAAATTGTGTTGACCGAGTCTCCAGGC**-3'

This primer corresponds to the 5' end of the KRIX-1 VL sequence (capital), and contains a *Xho*I restriction site (underlined) and a KEX1 sequence (bold italic);

reverse primer:

5'-cgccagagccacciccgcctgaaccgcctccacc**TCGTTTGATCTCCACCTTGGTC**

This primer corresponds to the 3' end of the KRIX-1 Jk sequence (capital), and contains a part of the linker sequence (italic).

b) heavy chain
forward primer:

5'-caggcggagggtggctctggcggggcggatcg**CAGGTMCAGCTGGTGCAGTCTGGG**-3'

This primer corresponds to the 5' end of the KRIX-1 VH sequence (capital), and contains a part of the linker sequence (italic);

reverse primer:

5'-gatctctaga**TGAGGAGACGGTGACCAGGGTTCC**

This primer corresponds to the 3' end of the KRIX-1 JH sequence (capital), and contains a *Xba*I restriction site (underlined).

The PCR products were annealed and a second PCR was performed using the forward primer for the light chain and the reverse primer for the heavy chain. The resulting scFv-KRIX-1VLVH was cloned into the pPICZαC expression vector (Invitrogen, Merelbeke, Belgium)

Bird Goën & Co

4

C1564-US

DRAFT-CONFIDENTIAL

2. Cloning of scFv-KRIX-1VLVH with His(6)tag in Pichia expression vector

A *SaI*I restriction site was added to the scFv-KRIX-1VLVH sequence in order to clone it in frame with the His(6) sequence included in the pPICZαC expression vector (Invitrogen; Merelbeke; Belgium). This was obtained by PCR using the forward primer 5'-gtatctctcgaagaaagaGAAATTGTGTTGACGCACTCTCCAGGC-3' corresponding to the 5' end of the KRIX-1 VL sequence (capital), and containing a *Xho*I restriction site (underlined) and a KEX1 sequence (bold italic); and the reverse primer 5'-batgggtogacTGAGGAGACGGTGACCAGGGTTCCCCGGCC-3' corresponding to the 3' end of the KRIX-1 heavy chain JH sequence (capital), and containing a *SaI*I restriction site (underlined).

The final pPICZαC-scFv-KRIX-1VLVH(His) vector was used to transform X33 cells for scFv production. The supernatant was tested to demonstrate the presence of a functional scFv fragment.

The scFv fragment was purified using the HisTrap Kit (Amersham Pharmacia Biotech, Uppsala, Sweden). After concentration the scFvKRIX-1VLVH(His) was tested in a FVIII chromogenic assay to evaluate the ability of the scFvKRIX-1VLVH(His) to inhibit FVIII activity. The FVIII inhibitory capacity was evaluated in a Besthesda assay.

TOTAL P.17

**BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM™
LMBP-COLLECTION**

Page 1 of Form BCCM™/LMBP/BP/4/98-14 Receipt in the case of an original deposit

**Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure**Receipt in the case of an original deposit issued pursuant to Rule 7.1 by the
International Depositary Authority BCCM™/LMBP identified at the bottom of next page**International Form BCCM™/LMBP/BP/4/99-14****To : Name of the depositor : JACQUEMIN MARC****Address : Center for Molecular and Vascular Biology
Onderwijs & Navorsing
Herestraat 49
3000 Leuven****I. Identification of the microorganism:****I.1 Identification reference given by the depositor:****KRIX 1****I.2 Accession number given by the International Depositary Authority:****LMBP 5089CB**

**BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM™
LMBP-COLLECTION**

Page 1 of Form BCCM™/LMBP/BP/4/89-14 Receipt in the case of an original deposit

**Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure**

Receipt in the case of an original deposit issued pursuant to Rule 7.1 by the
International Depositary Authority BCCM™/LMBP Identified at the bottom of next page

International Form BCCM™/LMBP/BP/4/89-14

To : Name of the depositor : JACQUEMIN MARC

Address : Center for Molecular and Vascular Biology
Onderwijs & Navorsing
Herestraat 49
3000 Leuven

I. Identification of the microorganism:

I.1 Identification reference given by the depositor:

KRIX 1

I.2 Accession number given by the International Depositary Authority:

LMBP 5089CB

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.